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Study of crude extract yield and gonad maturity level of sea cucumber *Phylloporus dobsoni* correlation

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Abstract. The study of the yield of n-hexane extract on the body wall of Phylloporus dobsoni sp. Is the first research conducted in Indonesia. This study aims to measure the n-hexane extract of the body wall of sea cucumber P. dobsoni which is related to the maturity level of the gonads. The measurement results obtained that the average wet weight and dry weight of sea cucumber body wall was 23.58 grams of body wall wet weight, body wall dry weight 5.56, gonad weight 4.02, and GSI value 18.03. Judging from the histology of male sea cucumber gonads, it is known that they have grown and grown at an advanced stage with an average content of n-hexane extract at an average value of 0.202 grams and 0.266 grams, respectively. Whereas in the female group there are all stages of growth, continued growth, maturity, recovery and post-spawning. The content of n-hexane extract showed an increase in the range of 0.085; 0.2506; 0.278; 0.354 and 0.3045. The increase in extract content in male and female sea cucumbers is possible as a protective mechanism from predation and protection in preserving offspring.

1. Introduction

Sea cucumber has habitat widespread throughout the world, ranging from tidal zone to the deep sea. About 10% of the number of sea cucumbers species in the world inhabit in Indonesian waters [1]. There more than 50 species commonly traded [2], and about 27 species of sea cucumbers identified as commercially potential derived from Indonesian waters [3]. In the international trade of sea cucumbers, 80% goes to Hong Kong, Indonesia and the Philippines leading supplier. Hong Kong exports of these products to China, Singapore and Taiwan [4]. In specific markets, found in raw form, usually used as a Chinese cuisine which is believed to have specific properties. In Surabaya and the surrounding areas, sea cucumbers are found in the form of ordinary snack, which is marketed in the form of fried sea cucumber [5].

According to the FAO global statistics on sea cucumber, Indonesia is the largest sea cucumber exporter in the world [6]. Recorded foreign exports of 2.100 tons of reef invertebrates out of which 320 tons were sea cucumber in 1981. The essential collecting and export centre for marine product trade-in Indonesia are Ujung Pandang in Sulawesi [7]. Researchers showed that the rate of Indonesian exports of dried sea cucumbers was massive, was not offset by the many useful products made of sea cucumbers from Indonesia. Food supplement products made from sea cucumbers mentioned have the benefits for health and healing [8], are imported, and no one has even mentioned that the material was essentially a sea cucumber in Indonesian waters.

Although the region is now the largest producer of sea cucumbers are the beaches in eastern Indonesia [1]. Similarly, this phenomenon occurred in other places in Indonesia [9]. It is not possible over the next ten years that the situation in eastern Indonesia suffers the same fate as the east coast of Surabaya if the rate of exploitation is not balanced with the preservation and cultivation efforts [6].

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Sea cucumber species collected in the study are not listed in the list of commercially valuable sea cucumbers in the world market. The distribution of those species are widespread, only one species with high abundance and distribution level, while others arranged from low to moderate. The preferred habitat is generally sandy. So far, *P. dobsoni* and *P. australis*are commonly consumed. Both are used for snacks or soup mix. The sea cucumbers are found, the type *P. dobsoni* has the highest abundance values comparing the other in the amount of 44.4%, and a high distribution rate is the distribution index of 1.9062 [13].

Based on a variety of the above information, it shows that the concern the development of species of *P. dobsoni* is still limited to the exploitation that is not sustainable. Based on morphological and molecular identification, this sea cucumber is *P. dobsoni* [10]. The attention that we paid in this study was an effort to develop cultivation level expected to be a developed laboratory and mass [5,11]. This study also constitutes active ingredients at the same owned *P. dobsoni* as a promising commodity in the future as pharmacological ingredients.

2. Material and methods

The sea cucumbers were purchased from local fisherman. They were collected from the east coastal water of Surabaya. The species of sea cucumber is *P. dobsoni* The sea cucumbers are performed surgery has not performed the selection, and the entire sample was 50 sea cucumbers. Each measurement of sea cucumbers wet weight, dry weight and the amount of the resulting extract. Besides, gonad weight and histology of gonads on each sea cucumber were done separately. Wet weight measurements were done using analytical digital scales. The body wall of sea cucumbers was weighing and cutting with a small size in order to facilitate the freeze-drying. The freeze-drying process is done for 24 hours at the Tropical Disease Center, Universitas Airlangga.

2.1. Gonadal histology

Gonadal histology performed by means of fixing the gonads in a liquid fixative neutral buffered formalin solution for ≥ 24 hours [12]. Furthermore, gonad histology has taken five of each gonad tubules for further processing from the processing stage, the stage of infiltration and planting (embedding), stage of cutting (sectioning) and attachment (affixing), and the last stage of staining (staining). Subsequent gonadal histology results were observed under a microscope with 100x magnification and documented.

2.2. Samples extraction

Extraction of sea cucumbers *P. dobsoni* done by maceration method that starts with the preparation of sea cucumbers material *P. dobsoni* obtained from the waters Kenjeran Beach, Surabaya. Sea cucumbers *P. dobsoni* discarded entrails and washed with running water. Samples that have been cleaned, put in a blender and then blended. The next process is freeze-drying so that the water content in the sample decreases but does not damage the existing active ingredients. Freeze-drying carried out at -80°C. Maceration process is done using hexane solvent [13]. Soaking the sample and the solvent in the ratio 1:3 (w/v) or submerged entirely. Soaking was done twice every 72 hours. Results filtrate formed filtering [14], then evaporated using a rotary evaporator at a temperature of 38^{o}C to form a viscous extract and have no smell of solvent [15]. The resulting extract yield calculation is done by calculating the percentage ratio of the resulting extract and extract raw materials, formulated in the following equation [14].

$$\text{Extract Yield (\%)} = (\text{weight extract} / \text{weight samples}) \times 100\%$$

2.3. Analysis of relationship gonads and hexane extract

Statistical analysis of the relationship and the yields of extract content based on gonad maturity level in each group of male and female sea cucumber do regression and graphs by Microsoft Excel [16]. From the result of the graph, the pattern extract content level can be obtained at each stage of gonad maturity both on sea cucumber: male and the female. The histology results are performed descriptively.

3. Result and discussion

The value of the yield of the crude extract (n-hexane) is about 0.242±0.233 grams or 4.35% of the dry weight obtained can be recognized. The dry weight of sea cucumber body wall by an average of 5.56±3.33 grams or about 23.57% of the average wet weight of 23,58±6.49 grams of the body wall (Table 1).

Table 1. Data of average weight wet, dry weight and the gonads weight of sea cucumber *P. dobsoni* and weight of n-hexane extract

Parameters	Value ± SD
Weight Wet (grams)	23.580 ± 6.490
Dry Weight (grams)	5.560 ± 3.330
Gonads Weight (grams)	4.020 ± 1.250
GSI	18.030 ± 7.870
n-Hexane Extract Weight (grams)	0.242 ± 0.233

Total results yield n-Hexane extract on sea cucumbers *P. dobsoni* This produced approximately 4.35% of the average weight of 5.56 grams of the dried body wall that produce an average weight of 0.242±0.233 gramsof extract. The dry weight of sea cucumber body wall by an average of 5.56 grams or about 23.57% of the average wet weight of 23.58 grams of the body wall. These conditions do not reflect the value of the content of other secondary metabolites that have not been tested in detail. This phenomenon is in contrast with the results of the yield on the previous study, which get *Stichopus variegatus* sea cucumber extract with methanol solvent, which is only 2.78% [14]. This results confirmed that the sea cucumber extract produced is very low, so if this is done will increase the value of selling sea cucumbers that can be used as efforts to increase value-added products of sea cucumbers.

At gonads maturity level (GML), the analysis on 43 samples of collected sea cucumbers known those 27 males, 13 females, and 3 samples are hermaphrodite. Seven samples are broken and unable to histological analysis. In the group of male sea cucumbers, the largest proportion of GML was at 66.67% growth stage, the advanced growth was 29.63%, and 3.7% was in the recovery phase (Figure 1).

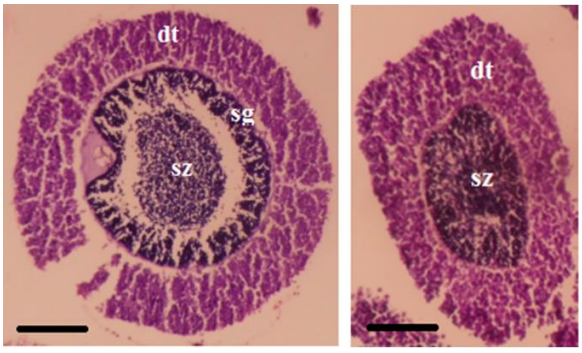


Figure 1. Histology of the gonads of sea cucumbers *P. dobsoni* males in the growth phase (A) and advanced growth (B). (dt: tubular wall; sz: spermatozoa; sg: spermatogonia) scale bar: 100μm with a magnification of 100 times.

In the group of female sea cucumbers, the highest proportion in the advanced stage of growth was 38.46%, 30.76% of it was in the mature stage, in the growth stage was 15.38% and the same percentage was in post-spawning stage and recovery of value which was 7.69% (Figure 4B). On histological analysis of the gonads, it was also found that there are three tails of sea cucumbers have two gonads (hermaphrodite), i.e. at the stage of growth, and advanced growth and recovery. The condition is known by the presence of spermatozoa (male marker) present in the gonads as well together as marker hermaphrodite oocytes (Figure 2).

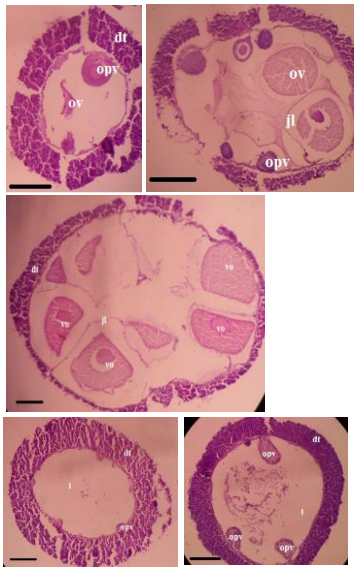


Figure 2. Histology of the gonads of sea cucumbers *P. dobsoni* females in the gonad development stage of growth (A), advanced growth (B), mature (C), post-spawning (D), and recovery (E). (dr: tubular wall; l: lumen; OPV: previtellogenic oocytes; ov: vitellogenin oocytes; jl: jelly layer) scale bar: 100μm and a magnification of 100 times.

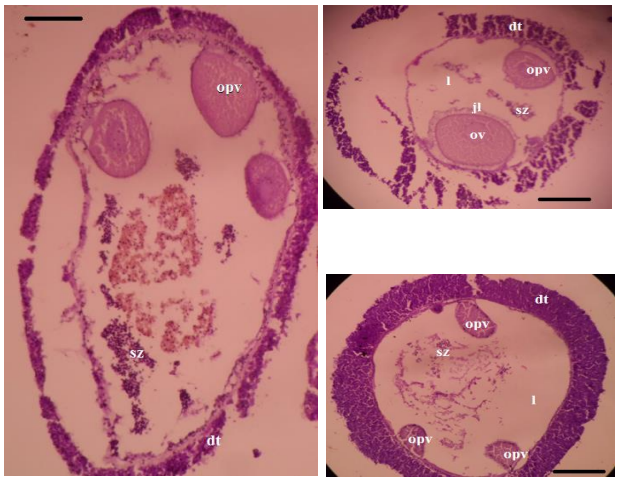
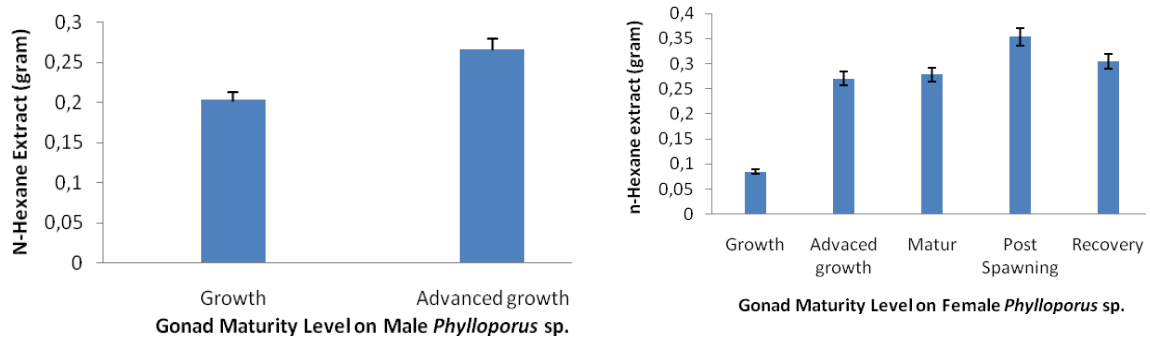


Figure 3. Gonadal histology *P. dobsoni* females who are hermaphroditic organisms on gonadal development stages of growth (A), advanced growth (B), and recovery (C) (dr: tubular wall; OPV: previtellogenesis oocytes; ov: vitellogenesis oocytes; sz: spermatozoa, l: lumen; and jl: jelly layer)) scale bar: 100 μm and a magnification of 100 times.

In the male gonads, histology results showed only at an advanced stage of growth and growth with a mean of n-hexane extract produced by 0.202 ± 0.1324 grams and 0.266 ± 0.3505 grams (Figure 4A). Values of n-hexane extract number increased 0.064 grams at earlier stages of gonad maturity. The biological activity of sea cucumbers *P. dobsoni* in the metabolism may produce results which are also focused on the improvement of future gonads to be generated.



(A) (B)

Figure 4. Graph content of n-Hexane extract the body wall *P. dobsoni* in the male group (A) and female (B) at different levels of gonadal maturity.

In the male group of sea cucumbers, found two stadia or the stage of maturity gonad growth and advanced growth. The second level of maturity gonad showed an increased amount of n-hexane extracts are produced. This condition proves that in the formation of the reproductive organs, sea cucumbers do protection mechanisms in the gonads produced in order to grow until the spawning stage. The secondary metabolites and organic acids in the body, internal organs and the gonads may play a role in self-defence. The active ingredients are also derived in the gonads and eggs, which allows preservation generation *Ascidians* group that studies [17]. This finding reinforces that sea cucumbers *P. dobsoni* is a benthic animal that enables very high-level predation because of its impaired mobility and has a soft body. This defence mechanism can be studied further in the content of secondary metabolites in the gonads that were not tested in the study this year.

Sea cucumber *P. dobsoni* females group are found throughout the stages of gonadal development of start growth, advanced growth, mature, post-spawning and recovery with the trend of increasing values of n-Hexane extract produced (Figure 4B). It also shows that the increase in future stages of gonad maturity of gonads will result in higher production of metabolites. This mechanism, both in males and females, occurs as a defence mechanism against other predators.

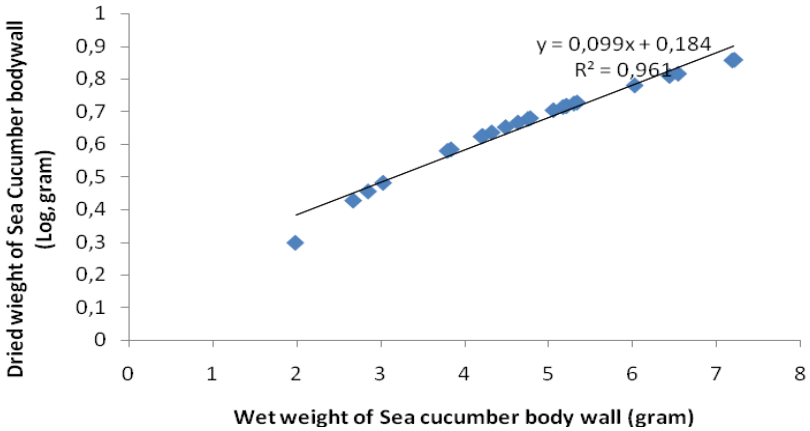


Figure 5. Regression chart between wet weight and dry weight of sea cucumbers

The graph above is the relationship between wet weight and dry weight of the body wall of sea cucumbers. The graph shows that the dry weight and wet weight has had a very close correlation between these parameters (Figure 5). This result shows that the weight of the sample in wet conditions, then dried samples obtained will also be more and more. The condition also occurs in relation to extracting obtained from the dry weight of the sample. The more dry samples are extracted, the more extracts that may be obtained. This finding can be seen in the graph below (Figure 6) with a correlation value of $R^2 = 0.909$.

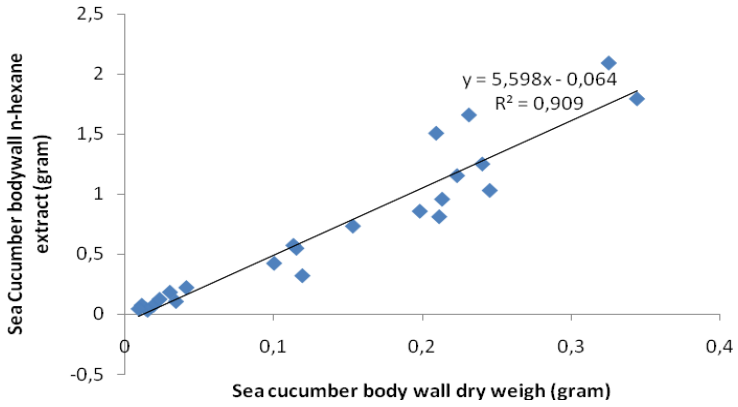
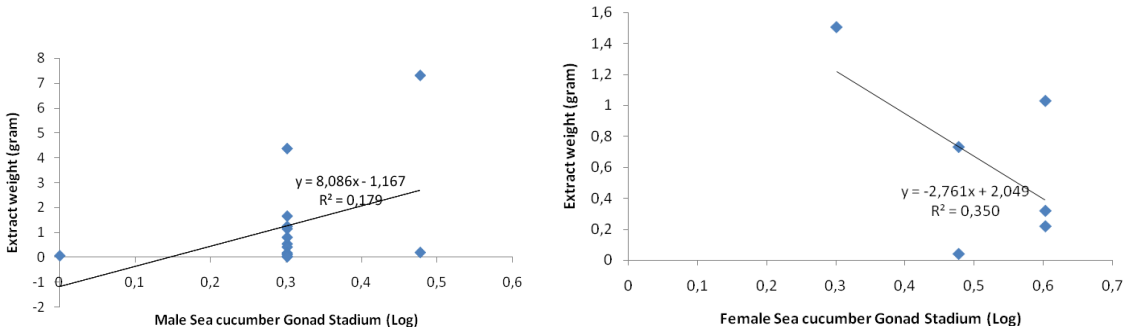


Figure 6. Regression graph dry weight of sea cucumbers and n-hexane extracts yield.



A B

Figure 7. Regression graph of gonads maturity stadia and yields extract in males (A) and female (B)

Regression graph above illustrates that the content of the extract is not related to the maturity level of gonads, either male or female in the sea cucumber (Figure 7). Although the extract obtained is useful metabolites in protection, but does not affect the maturity of gonads of sea cucumbers. Internal factors of sea cucumbers do not only influence the maturity of gonads of sea cucumbers, but external factors are also influential. Many environmental factors are thought to affect the reproductive aspects of sea cucumbers, including temperature, blooming phytoplankton, and the cycles of the moon [18].

4. Conclusion

The number of natural products produced from the body wall extract *P. dobsoni* with n-Hexane solvent is an average of 0.242±0.233 grams or 4.35% of the dry weight obtained. The dry weight of sea cucumber body wall by an average of 5.56 grams or about 23.57% of the average wet weight of 23.58 grams of the body wall. Thus, the content of n-Hexane extract yield in the body wall *P. dobsoni* can be estimated by referring to this study. The correlation between wet weight and dry weight indicates a very close value. This result means that the weight of the body wall of sea cucumbers, the more will allow the amount of dry weight was obtained. The amount of the extract obtained also has a linear pattern on the dry weight. The regression equation concerning the dry weight of the body wall and extract the resulting number is Y = 0,099X + 0.184, with the value of x is the dry weight of the body wall of sea cucumbers and y as the number of n-hexane extracts are produced.

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